

REMARKS

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A unexecuted DECLARATION under 37 C.F.R. §1.132 of Perez is attached hereto. The executed original DECLARATION will be submitted upon receipt.

The specification has also been amended to correct obvious typographical errors. Therefore no new matter has been added by the amendment.

Claims 32-44, 59, 60 and 64-81 are presently pending in this application. Claims 1-31, 45-58 and 61-63 have been cancelled without prejudice herein as being drawn to non-elected subject matter. Applicant reserves the right to file divisional applications to the non-elected subject matter. Claims 32, 35-44, 59 and 60 have been amended and claims 64-81 have been added in order to more particularly point out and distinctly claim the subject matter that applicant regards as the invention. No amendments have been made to obviate the prior art and no new matter has been introduced. The amendments to claims 32, 35-44, 59 and 60 find basis in the specification and claims as originally filed. Therefore, since the amendments change the form, not the substance of the claimed subject matter, no new matter has been added. Accordingly, entry of the amendments to the claims is respectfully requested.

Claims 64-81 have been added. These claims find basis in the specification as originally filed. For example, claims 64-66 and 68-72 find particular basis at page 34, lines 2-5 and 15-31, page 109, lines 13-21; page 111, lines 4-14. New claim 67 finds basis in the specification, for example, at page 27, lines 5-12 and page 44, lines 18-22. Basis for claims 73-79 may be found, for example, on page 6, lines 13-17; page 22, line 17, through page 23, line 4; page 29, lines 20-30; and on page 67, lines 3-8. Basis for claims 80-82

may be found, for example, on page 3, lines 25-26; page 9, lines 9-10 and 27-29; page 38, lines 8-11; and page 106, lines 8-9. Because the new claims find basis in the specification and the claims as filed, no new matter has been added. Accordingly, entry of the new claims in the case is respectfully requested.

Claims 1-31, 45-58 and 61-63 have been cancelled without prejudice.

THE REJECTION OF CLAIM 42 UNDER 35 U.S.C. §101

Claim 42 is rejected under 35 U.S.C. §101 as allegedly directed to non-statutory subject matter because it encompasses a human being. It was suggested by the Examiner in the Office Action that recitation of the term "non-human" would be remedial. Claim 42 has been amended to include the term "non-human."

THE REJECTION OF CLAIMS 42-44, 59 and 60 UNDER 35 U.S.C. §112, FIRST PARAGRAPH

Claims 42-44, 59 and 60 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which allegedly is not described in the specification in such a way as to enable one skilled in the art to make and/or use the invention. It appears that the Examiner may have intended this rejection to apply to claims 32-41 in addition to the claims (i.e., 42-44, 59 and 60) as set forth in the statement of the rejection on page 5 of the Office Action.

Specifically, it is asserted that because the production of transgenic animals by means of mammalian artificial chromosomes (MACs) is not well established in the art, the artisan must rely on the specification for guidance as to how to make and use MACs in this manner, and the specification allegedly does not teach (1) how to generate MACs that contain and express heterologous genes, (2) how to introduce MACs into embryos or embryonic stem (ES) cells, or (3) how to generate transgenic animals which express heterologous genes at a high enough level to be useful. Reconsideration of these grounds for the rejection is respectfully requested based on the following remarks.

Relevant law

In order to satisfy the enablement requirement of 35 U.S.C § 112, first paragraph, the specification must teach one of skill in the art to make and use the invention without undue experimentation. Atlas Powder Co. v. E.I. DuPont de Nemours, 750 F.2d 1569, 224 USPQ 409 (1984). This requirement can be satisfied by providing sufficient disclosure, either through illustrative examples or terminology, to teach one of skill in the art how to make and how to use the claimed subject matter without undue experimentation. This clause does not require "a specific example of everything *within the scope* of a broad claim." In re Anderson, 176 USPQ 331, at 333 (CCPA 1973), emphasis in original. Rather, the requirements of § 112, first paragraph "can be fulfilled by the use of illustrative examples or by broad terminology." In re Marzocchi et al., 469 USPQ 367 (CCPA 1971)(emphasis added).

The inquiry with respect to scope of enablement under 35 U.S.C. § 112, first paragraph, is whether it would require undue experimentation to make and use the claimed invention. A considerable amount of experimentation is permissible, particularly if it is routine experimentation. The amount of experimentation that is permissible depends upon a number of factors, which include: the quantity of experimentation necessary, the amount of direction or guidance presented, the presence or absence of working examples, the nature of the invention, the state of the prior art, the relative skill of those in the art, the predictability of the art, and the breadth of the claims (i.e. the "Forman factors"). Ex parte Forman, 230 USPQ 546 (Bd. Pat. App. & Int'f 1986); see also In re Wands, 8 USPQ2d 1400 (Fed. Cir. 1988).

It would not require undue experimentation to practice the claimed methods and make and use the claimed transgenic animals.

As discussed below, the claims are commensurate in scope with the disclosure, which exemplifies particular embodiments within the scope of the claims and also teaches how one of skill in the art can obtain other

embodiments within the scope of the claims. In particular, there is an enormous amount of guidance presented in the specification, there are numerous working examples, the level of skill in the art is high, and the state of the prior art at the time of filing of the application was such that a large amount information concerning recombinant DNA techniques and procedures for the manipulation of DNA was available. Therefore, it would not require undue experimentation for one of skill in the art to make and use the claimed subject matter.

Evaluation of the above Factors

1. The scope of the claims

Claims 32-41, 43, 44, 59 and 60 are directed to methods of making transgenic animals by introducing a satellite artificial chromosome or a minichromosome generated by a prescribed method into an animal cell which is used in the generation of a transgenic animal. Claim 42 is directed to a non-human transgenic animal produced by a method comprising introducing a satellite artificial chromosome into an embryonic cell.

All of the claims are directed to methods and compositions that incorporate satellite artificial chromosomes or minichromosomes. The specification describes in extensive detail the preparation, characterization and isolation of artificial chromosomes, and in particular satellite artificial chromosomes and minichromosomes, and provides numerous examples of particular embodiments thereof. The specification further describes methods of incorporating heterologous DNA into MACs, expression of the heterologous DNA in cells containing MACs and the use of such MACs in the preparation of transgenic animals.

Applicant is entitled to claims that are commensurate in scope not only with what applicant has specifically exemplified, but commensurate in scope with that which one of skill in the art could obtain by virtue of that which the applicant has disclosed. In the above-captioned application, Applicant discloses to the public methods and compositions for the controlled introduction and

stable extra-genomic maintenance of large heterologous DNA fragments in cells without disruption of the inherent genome and, likewise, without the otherwise uncontrollable influences that the genomic DNA may have on the expression of the heterologous DNA. The artificial chromosomes disclosed in the application can be manipulated and used to express heterologous genes in cells, as is taught and specifically exemplified in the specification. It is clear that Applicant's discovery is of a pioneering nature, and, as such, is entitled to broad claim protection.

As taught in the above-captioned application, any methods known in the art pertaining to introduction of foreign genes carried in traditional, standard sources (such as genes harbored in expression vectors) into cells for any variety of purposes, e.g., gene therapy, protein production and the generation of transgenic animals, may be applied in similar fashion to the introduction of MACs, particularly SATACs and minichromosomes, into cells. The application describes and demonstrates that once the artificial chromosomes are generated and isolated and/or introduced into cells, then any known procedure that has previously been carried out with any heterologous gene from any source is applicable to utilization of artificial chromosomes carrying foreign genes of interest. The application is replete with descriptions of numerous uses of SATACs and minichromosomes. The descriptions of the many ways in which the artificial chromosomes may be used include references to reported procedures for introducing exogenous nucleic acids into cells.

It is therefore respectfully submitted that the claims directed to methods of producing transgenic animals using SATACs and minichromosomes are commensurate in scope with the discovery and its disclosure within the above-captioned application. It would be unfair and contrary to the Constitutional mandate set forth in Article, Section 8, to deprive Applicant of protection of the broad applications of the pioneering discovery disclosed and described in exhaustive detail in the subject application.

2. Level of skill

The level of skill in this art is recognized to be high (see, *e.g.*, Ex parte Forman, 230 USPQ 546 (Bd. Pat. App. & Int'f 1986)). The numerous articles and patents made of record in this application, authored and reviewed by those known in the art, further evidences the high degree of skill in this art.

3. State of the prior art

At the time of filing of the application, a broad body of knowledge had amassed in the area of molecular biology including many technical procedures covering the manipulation of DNA and recombinant DNA techniques. Numerous such procedures are referenced in the instant application. For example, procedures for the introduction of DNA into cells are referred to in many instances throughout the application:

— on page 8, lines 8-19, the application describes that introduction is effected by any suitable method including, but not limited to electroporation, direct uptake, such as by calcium phosphate [see, *e.g.*, Wigler et al. (1979) Proc. Natl. Acad. Sci. U.S.A. 76:1373-1376; and Current Protocols in Molecular Biology, Vol. 1, Wiley Inter-Science, Supplement 14, Unit 9.1.1-9.1.9 (1990)], precipitation, uptake of isolated chromosomes by lipofection, by microcell fusion [see, EXAMPLES, see, also Lambert (1991) Proc. Natl. Acad. Sci. U.S.A. 88:5907-5911, U.S. Patent No. 5,396,767] or other suitable method;

— on page 32, lines 1-15, the application refers to other methods for introducing DNA into cells, which include nuclear microinjection, electroporation, bacterial protoplast fusion with intact cells... polycations, such as polybrene and polyornithine, may also be used. For various techniques for transforming mammalian cells, see *e.g.*, Keown et al. Methods in Enzymology (1990) Vol. 185, pp. 527-537; and Mansour et al. (1988) Nature 336:348-352. DNA may be introduced by direct DNA transformation; microinjection in cells or embryos, protoplast regeneration for plants, electroporation, microprojectile gun and other such methods [see, *e.g.*, Weissbach et al. (1988) Methods for Plant Molecular

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Biology, Academic Press, N.Y., Section VIII, pp. 421-463; Grierson et al. (1988) Plant Molecular Biology, 2d Ed., Blackie, London, Ch. 7-9; see, also U.S. Patent Nos. 5,491,075; 5,482,928; and 5,424,409; see, also, e.g., U.S. Patent No. 5,470,708, which describes particle-mediated transformation of mammalian unattached cells];

— on page 33, lines 1-5, of the application, methods of DNA uptake are further described with reference to published procedures: for mammalian cells without such cell walls, the calcium phosphate precipitation method [see, e.g., Graham et al. (1978) Virology 52:456-457 is often preferred. DNA uptake can be accomplished by DNA alone or in the presence of polyethylene glycol [PEG-mediated gene transfer], which is a fusion agent, with plant protoplasts or by any variations of such methods known to those of skill in the art [see, et al. U.S. Pat. No. 4,684,611];

— on page 33, lines 14-22, of the application, DNA transfer procedures based on electroporation are further referenced: methods for effecting electroporation are well known [see, e.g., U.S. Patent Nos. 4,784,737, 5,501,967, 5,501,662, 5,019,034, 5,503,999; see, also Frommet et al. (1985) Proc. Natl. Acad. Sci. U.S.A. 82:5824-5828], for example, electroporation is often used for transformation of plants [see, e.g., Ag Biotechnology News, Vol. 7 p. 3 and 17 (September/October 1990)];

— on page 34, lines 5-11, the application provides further references to DNA transfer procedures based on microcell fusion: the chromosomes can be transferred by preparing microcells containing an artificial chromosome and then fusing with selected target cells... methods for such preparation and fusion of microcells are well known [see, e.g., U.S. Patent Nos. 5,240,840, 4,806,476, 5,298,429, Fournier (1981) Proc. Natl. Acad. Sci. U.S.A. 78:6349-6353; and Lambert et al. (1991) Proc. Natl. Acad. Sci. U.S.A. 88:5907-59];

— on page 43, lines 13-15 and lines 25-30, further references to cell fusion methods are provided as follows: mouse and hamster cells were fused

using polyethylene glycol [Davidson et al. (1976) Som. Cell Genet. 2:165-176)] and microcell-mediated chromosome transfer was done according to Saxon et al. [(1985) Mol. Cell. Biol. 1:140-146] with the modifications of Goodfellow et al. [(1989) Techniques for mammalian genome transfer. In *Genome Analysis a Practical Approach*. K.E. Davies, ed., IRL Press, Oxford, Washington DC. pp.1-17] and Yamada et al. [(1990) Oncogene 5:1141-1147].

Procedures for the introduction of DNA into cells for the production of transgenic animals are also referred to in numerous instances throughout the application:

— on page 34, line 29, through page 35, line 14, the application refers to several published methods of introducing DNA into embryos, for example, transgenic animals can be produced by introducing exogenous genetic material into a pronucleus of a mammalian zygote by microinjection [see, e.g., U.S. Patent Nos. 4,873,191 and 5,354,674; see, also, International PCT application No. WO95/14769, which is based on U.S. application Serial No. 08/159,084]. DNA can be introduced into animal cells using any known procedure, including, but not limited to: direct uptake, incubation with polyethylene glycol [PEG], microinjection, electroporation, lipofection, cell fusion, microcell fusion, particle bombardment, including microprojectile bombardment [see, e.g., U.S. Patent No. 5,470,708, which provides a method for transforming unattached mammalian cells via particle bombardment], and any other such method. For example, the transfer of plasmid DNA in liposomes directly to human cells *in situ* has been approved by the FDA for use in humans [see, e.g., Nabel, et al. (1990) Science 249:1285-1288 and U.S. Patent No. 5,461,032];

— on page 107, lines 25-30, the application provides a reference for embryo microinjection procedures: the microinjection procedure used to introduce the plasmid DNA into the mouse embryos is as described in Example 13, but modified for use with embryos [see, e.g., Hogan et al. (1994) *Manipulating the Mouse Embryo, A Laboratory Manual*, Cold Spring Harbor

Laboratory Press, Cold Spring Harbor, NY, see, especially pages 255-264 and Appendix 3].

Procedures for the generation of transgenic animals are also referred to throughout the application:

— on page 108, lines 16-19, the application provides a reference for the culture and transfer of embryos that have received foreign DNA: [for procedures see, Manipulating the Mouse Embryo, A Laboratory Manual (1994) Hogan et al., eds., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp. 127 et seq.];

— on page 110, lines 11-21, yet another reference is provided for procedures relating to preparation of zygotes and injection of male pronuclei with DNA: [see, e.g., Manipulating the Mouse Embryo, A Laboratory Manual (1994) Hogan et al., eds., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, p. 429];

— on page 111, lines 11-18, references to cell fusion-based methods for introduction of DNA into embryonic cells are provided: [see, e.g., U.S. Patent No. 5,453,357, commercially available; see Manipulating the Mouse Embryo, A Laboratory Manual (1994) Hogan et al., eds., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pages 253-289] following standard procedures see also, e.g., Guide to Techniques in Mouse Development in Methods in Enzymology Vol. 25, Wassarman and De Pamphilis, eds. (1993), pages 803-932].

These references to numerous published protocols for DNA manipulation and recombinant DNA expression demonstrate the large volume of information regarding tested and reliable procedures available at the time of filing of the instant application and thus evidence the advanced state of the art at the relevant time.

4. The amount of direction and guidance presented, and teachings in the specification

The claims are directed to methods of producing transgenic animals in which SATACs or minichromosomes are introduced into animal cells that are then used in the generation of the transgenic animal.

The specification discloses methods of generating artificial chromosomes, such as SATACs and minichromosomes, and characterizes in exquisite detail the artificial chromosomes generated by such methods. To illustrate the methods and products thereof, the specification describes the exact procedures used repeatedly to generate multiple specific cell lines containing MACs (see Examples 2-7), and the Applicant provides to the public no less than six of the described cell lines which have been deposited at an authorized depository (i.e., the European Collection of Animal cell Culture) (see page 45, lines 6-11).

The MACs contained within these cells were extensively characterized using methods including Southern hybridization, long-range mapping of restriction endonuclease sites, indirect immunofluorescence with anti-centromere antibodies, in situ hybridization, analysis of G-band patterns, and chromosome painting. Such intensive analysis enabled definition of the MACs at the level of the basic structural and functional elements that comprise these chromosomes, including the characteristic repeated units of satellite and foreign DNA. Many of these features are depicted schematically in Figures 1-3 of the application.

The specification further teaches methods of inserting heterologous DNA into the artificial chromosomes and the expression of the heterologous DNA contained therein in cells (see, e.g., page 22, line 25, through page 23, line 29; page 30, lines 1-23; page 58, lines 11-23 and Example 12 beginning on page 85). Procedures for the isolation of artificial chromosomes (see, e.g., page 23, lines 13-22; page 31, lines 16-20; page 49, line 25, through page 50, line 3; and Example 10, beginning on page 79) and for the transfer of the artificial

chromosomes into cells (see, e.g., page 8, lines 10-17; page 9, lines 1-4; page 34, lines 5-11; page 34, line 26, through page 37, line 8; page 42, line 12, through page 43, line 8; and page 50, line 28, through page 51, line 12) are also described in detail in the specification.

Additionally, the specification provides multiple possible uses of the artificial chromosomes with reference to procedures involved in those applications where appropriate. For example, the specification teaches on page 4, lines 19-24, that artificial chromosomes with integrated heterologous DNA may be used in methods of gene therapy, in methods of production of gene products, particularly products that require expression of multigene biosynthetic pathways, and also are intended for delivery into the nuclei of germline cells, such as embryo-derived stem cells [ES cells] for production of transgenic animals. The specification further teaches on page 8, lines 23-30, that artificial chromosomes can be used in the production of humanized organs, transgenic plants and animals, including invertebrates, vertebrate, reptiles and insects, any organism or device that would employ chromosomal elements as information storage vehicles, and also for analysis and study of centromere function, for the production of artificial chromosome vectors that can be constructed in vitro, and for the preparation of species-specific artificial chromosomes. Particular methods for the production of transgenic animals with cells that contain artificial chromosomes are described, for example, on page 34, line 26, through page 35, line 14, and Example 14, beginning on page 107.

In summary, the specification enables one of ordinary skill in the art to, by following the methods set forth therein, generate artificial chromosomes, readily identify the resulting artificial chromosomes based on the detailed characterization provided in the specification, incorporate foreign DNA into an artificial chromosome, and isolate and transfer artificial chromosomes for use in other cells and systems. By virtue of Applicant's discovery of artificial chromosomes and the teachings of the specification, those of ordinary skill in

the art are able, without undue experimentation, to make and use the artificial chromosomes and to combine the artificial chromosomes with known recombinant DNA procedures, many of which are referenced in the specification, to achieve any number of particular outcomes, including the generation of transgenic animals.

5. Nature of the invention

Clearly, Applicant's discovery of a means of producing synthetic chromosomes, the basic functional units common to all eukaryotes for the storage and transmission of vital genetic information, which are maintained extra-genomically in host cells but function in the same manner as endogenous chromosomes, has broad and immediate applicability in the field of recombinant DNA. Applicant is entitled to claims of a scope commensurate with the far-reaching development which has been provided to the public for immediate and valuable use through the guidance of the instant specification.

Rebuttal of the specific issues raised in the office action.

The generation of MACs that comprise and express heterologous genes

On page 7 of the Office Action mailed August 17, 1999, the Examiner summarizes some of the multiple forms of MACs disclosed in the application. As a preliminary matter, Applicant notes an error in the characterization of the neo-centromere of the dicentric chromosomes in cell line EC3/7C5 as originating from human chromosome 7. In fact, as described in the subject application at page 21, lines 23-24, the neo-centromere of this cell line is derived from mouse chromosome 7.

It is asserted in the Office Action that there is no indication in the specification that MACs can be obtained in a reproducible fashion or that centromere amplification can occur in other mammalian chromosomes or centromeres from other species; therefore, it is alleged that, given the lack of understanding of the structure and function of centromeres, coupled with the

rare occurrence of MACs, the only MACs whose derivation is enabled are those which are exemplified in the specification and which have been deposited. In addition, it is asserted that there is no evidence that the MACs can be used as a basis for introducing and expressing foreign genes in animals. Applicant respectfully disagrees with these grounds for rejection of the claimed subject matter under 35 U.S.C. §112, first paragraph, for the following reasons.

1. **The method of generation of MACs is based in a consistent, broadly applicable process and is reproducible as demonstrated by production of a MAC from mouse chromosome 1 and the elucidation of the mechanism of replication of MACs.**

First, the specification does provide examples (see Example 9 beginning on page 77 and Example 8 beginning on page 68) of the reproducibility and wide applicability of the method of generating artificial chromosomes provided therein.

(a) Example 9 of the instant application

Example 9 describes an independent transformation experiment in which a selectable puromycin construct pPuroTel (i.e., an entirely different construct and selectable marker than that used in the generation of MACs in cell line EC3/7) was introduced into an LMTK⁻ cell line to generate the LP11 cell line. This cell line carries chromosome(s) with amplified chromosome segments of different lengths [~150-600 Mb]. Cytological analysis of the LP11 cells indicated that the amplification occurred in the pericentric region of the long arm of a submetacentric chromosome identified by G-banding as mouse chromosome 1. C-banding and *in situ* hybridization with mouse major satellite DNA probe showed that an E-type amplification had occurred.

This Example demonstrates a key feature of the method described in the specification for generation of artificial chromosomes. It is most significant that the amplification that gave rise to the artificial chromosome in the LP11 cell line occurred in the *pericentric* region of chromosome 1. This result demonstrates

that the generation of artificial chromosomes via the methods described in the application proceeds consistently through the same mechanism of amplification of DNA in the pericentric region of the chromosome. Eukaryotic genomes include chromosomes that contain pericentric heterochromatin, and all such chromosomes carry out the same basic functions, such as replication, in similar fashion. Thus, absent any evidence to the contrary, there is no reason to believe that a method that involves amplification of this region of the chromosome would not be broadly applicable and reproducible. Clearly, in this specific example, the fact that amplification occurred on chromosome 1 reveals that the method is not limited to generation of MACs from mouse chromosome 7. The results described in Example 9 of the application also demonstrate the ability to use various sources of heterologous DNA and selectable marker genes in the method described in the application for the generation of artificial chromosomes.

Additionally, it was possible to determine that the initial amplification that occurred in the generation of a MAC from mouse chromosome 1 was an E-type amplification, as was the case in the generation of MACs from mouse chromosome 7. This finding supports Applicant's assertion that the method described in the application is a reproducible method that proceeds in a consistent manner for the generation of artificial chromosomes from many different chromosomes. Thus, there is no indication that the H-type amplifications that occurred in EC3/7C5 cells to generate satellite artificial chromosomes from the formerly dicentric chromosome 7 therein would not also occur in the LP11 cell line subjected to the methods described in the application. Therefore, in view of Example 9, there is no basis for asserting that there is no indication that artificial chromosomes can be obtained in a reproducible fashion using the methods described in the application.

(b) Example 8 of the instant application

As described in detail in the above-captioned application (see e.g., page 20, lines 6-14 and Example 8), Applicant's method of artificial chromosome production is based in an amplification-driven process that can be induced by integration of exogenous nucleic acids into the pericentric region of a chromosome. Exhaustive analysis and characterization of artificial chromosomes produced by this method, the results of which are described in the instant application, revealed the existence of a higher-order replication unit [megareplicon] of the pericentric region. The megareplicon is delimited by a primary replication initiation site [megareplicator], and appears to facilitate replication of the centromeric heterochromatin, and most likely, centromeres. Integration of heterologous DNA into the megareplicator region or in close proximity thereto, initiates a large-scale amplification of megabase-size chromosomal segments, which leads to *de novo* chromosome formation.

Example 8 (beginning on page 68) of the application describes the methods and results of BrdU pulse labelling and immunolabelling experiments conducted on H1D3 mouse-hamster hybrid cells carrying a megachromosome, and mouse A9 cells for comparative purposes, in order to study the replication of the megachromosome. From these studies, the sequence of replication in the megachromosome was determined to be as follows. At the very beginning of the S-phase, the replication of the megachromosome starts at the ends of the chromosome. The first initiation of replication in an interstitial position can usually be detected at the centromeric region. Soon after, but still in the first quarter of the S-phase, when the terminal region of the short arm has almost completed its replication, discrete initiation signals appear along the chromosome arms. In the second quarter of the S-phase, as replication proceeds, the BrdU-labelled zones gradually widen, and the checkered pattern of the megachromosome becomes clear [see, e.g., Fig. 2F of the instant application]. At the same time, pericentric regions of the endogenous mouse

chromosomes also show intense incorporation of BrdU. The replication of the megachromosome peaks at the end of the second quarter and in the third quarter of the S-phase. At the end of the third quarter, and at the very beginning of the last quarter of the S-phase, the megachromosome and the pericentric heterochromatin of the endogenous mouse chromosomes complete their replication.

Similar analyses of the replication in mouse A9 cells were performed as controls. On those chromosomes where the initiation signal(s) were localized unambiguously, the replication of the pericentric heterochromatin of A9 chromosomes was similar to that of the megachromosome. Chromosomes of A9 cells also exhibited replication patterns and sequences similar to those of the mouse chromosomes in the hybrid cells. These results indicate that the replicators of the megachromosome and mouse chromosomes retained their original timing and specificity in the hybrid cells.

To identify origins of replication (initiation sites) in relation to the amplicon structure of the megachromosome, the pattern of the initiation sites obtained after BrdU incorporation was compared with the location of the integration sites of the foreign DNA in a detailed analysis of the first quarter of the S-phase. The results showed a colocalization of the BrdU and *in situ* hybridization signals found at the cytological level, indicating that the foreign DNA sequences are in close proximity to the origins of replication, presumably integrated into the non-satellite sequences between the replicator and the satellite sequences [see Figure 3 of the instant application]. Similar initiation signals were observed at the same time in the pericentric heterochromatin of mouse chromosomes that do not have "foreign" DNA, indicating that the replication initiation sites at the borders of amplicons may reside in the non-satellite flanking sequences of the satellite DNA blocks.

The finding that a specific replication initiation site occurs at the boundaries of amplicons where the foreign DNA integrated and triggered amplification leading to MAC formation indicates that replication plays a role in the amplification process. A replication-directed mechanism thus may form the basis for the initiation of large-scale amplifications in the centromeric regions of mouse chromosomes, as well as for the *de novo* chromosome formation giving rise to artificial chromosomes disclosed in the instant application.

All eukaryotic chromosomes must undergo replication in order for both of the cells resulting after cell division to possess the full complement of DNA that was contained in the parent cell. As demonstrated in the experiment described in Example 8, there was a similarity of replication initiation signals in the megachromosome and endogenous mouse chromosomes in H1D3 cells. These primary origins of replication within the pericentric heterochromatin were determined to be at the borders of each amplicon of the megachromosome. Furthermore, the chromosomes of mouse A9 cells also exhibited replication patterns and sequences similar to those of the megachromosomes indicating that the replicators of the megachromosomes retained their original timing and specificity.

These results and the mechanism elucidated therefrom provide evidence that Applicant's method for *de novo* formation of artificial chromosomes is widely applicable to other chromosomes. All chromosomes undergo replication which is thought to universally proceed via initiation at certain origins within the chromosome. Applicant's analysis provided in Example 8 reveals that there are primary replication initiation sites in the pericentric region of chromosomes that provide for replication of the pericentric heterochromatin and likely centromeric DNA. The colocalization of the site of integration of foreign DNA in megachromosomes, the event that induces *de novo* artificial chromosome formation, with the site of replication initiation reveals that Applicant's amplification-based method of generating artificial chromosomes involves a

replication system inherent in chromosomes. Accordingly, it should be possible, as set out in Applicant's method for generating artificial chromosomes, for integration of exogenous DNA into the pericentric region of any chromosome in which the pericentric DNA is replicated via initiation at origins of replication in that region to lead to the de novo formation of artificial chromosomes.

It should be further noted that in order to practice the claimed methods of producing transgenic animals, it is not necessarily required that the artificial chromosomes used in the methods are specific to the host species. For example, there is ample evidence that vertebrate chromosomes from one species are functional in cells from another species. Each of the 23 human chromosomes has been successfully transferred into mouse or hamster cells. In these monochromosomal hybrids, the human chromosomes are maintained and are functional. Furthermore, it has been shown that an enzyme (i.e., hypoxanthine phosphoribosyltransferase or HPRT) deficiency in certain hamster cells can be successfully complemented with chicken chromosomes carrying the HPRT gene [see, e.g., Klinger et al. (1974) Proc. Natl. Acad. Sci. U.S.A. 71: 1398-1402 and Rasko et al. (1979) Cytogenet. Cell Genet. 24:129-137, copies of which are provided herewith]. In addition, the application, on page 67, describes the 19C5xHa4 cell line which is a mouse-hamster hybrid containing the mouse chromosome-derived sausage chromosome carrying both neomycin and hygromycin resistance genes. The ability of this mouse-derived MAC to function in a hamster cell environment is demonstrated by the ability of 19C5xHa4 cells to survive on plates containing G418 and hygromycin. Accordingly, even if a species-specific artificial chromosome was not available for use in the generation of a transgenic animal of a particular species, it is entirely possible that an artificial chromosome based in the DNA of a different species would be functional in the desired transgenic animal species.

2. **The level of understanding of centromere structure and function and the level of occurrence of MACs are irrelevant to a determination of whether undue experimentation is required to practice the methods of generating artificial chromosomes.**

When rejecting a claim under the enablement requirement of 35 U.S.C. §112, "the PTO bears an initial burden of setting forth a reasonable explanation as to why it believes that the scope of protection provided by [the] claim is not adequately enabled by the description of the invention provided in the specification of the application; this includes, of course, providing sufficient reasons for doubting any assertions in the specification as to the scope of enablement." In re Wright, 999 F.2d 1557, 1562, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993). The nature of this burden is further elucidated in In re Marzocchi (439 F.2d 220, 223, 169 USPQ 367, 369 (CCPA 1971)): "[I]t is incumbent upon the Patent Office, whenever a rejection on this basis is made, to explain why it doubts the truth or accuracy of any statement in a supporting disclosure and to *back up assertions of its own with acceptable evidence or reasoning* which is inconsistent with the contested statement. Otherwise, there would be no need for the applicant to go to the trouble and expense of supporting his presumptively accurate disclosure" (emphasis added).

It is respectfully submitted that the reasoning (as no evidence is provided) offered on pages 7 and 8 of the Office Action in connection with the grounds asserted for rejecting the instant claims under 35 U.S.C. §112, first paragraph, fails to meet the burden as set forth in the cited cases. It is alleged in the Office Action that given the lack of understanding of the structure and function of centromeres, coupled with the rare occurrence of the MACs, the only MACs, minichromosomes or SATACs whose derivation is enabled are those which are exemplified in the specification and which have been deposited. It is respectfully submitted that this reasoning is invalid for the following reasons.

First, the level of understanding of the structure and function of centromeres is not relevant to the question of whether Applicant's method for generating artificial chromosomes and the artificial chromosomes generated thereby are enabled for embodiments other than those exemplified by the deposited cell lines referred to in the specification. As described above, the method for generating artificial chromosomes disclosed in the specification is based on an amplification process that occurs within the pericentric region of the chromosome and is independent of centromere structure or function. Therefore, the method for generating artificial chromosomes disclosed by Applicant in the above-captioned application does not rely on an understanding of the structure and function of centromeres.

Second, in alleging that MACs are a "rare occurrence," presumably the Examiner is referring to the number of cell lines analyzed in identifying those that contain artificial chromosomes as recited on page 7 of the Office Action. Specifically, the Examiner cites the post-filing publication of Kereso et al. [(1996) Chromosome Res. 4:226-239] in stating that a sausage chromosome occurred once in 62 cell lines analyzed and a gigachromosome occurred once in 27 cell lines analyzed. It is noted that the Examiner did not further cite the occurrence of the megachromosome as reported in Kereso et al. which states that 42 out of 79 subclones carried an intact megachromosome. Nonetheless, Applicant respectfully disagrees with the interpretation of any of these occurrences as "rare." Furthermore, even if the occurrence of artificial chromosomes generated by the methods described in the instant application could be characterized as "rare," this is not the proper standard to be used in determining whether a disclosure meets the enablement requirements of 35 U.S.C. §112, first paragraph. As stated above, the proper standard is whether it would require undue experimentation for one of skill in the art to make and use the invention.

The Court of Appeals for the Federal Circuit has recognized that screening of cell lines is a standard procedure in the field of biotechnology that does not necessarily constitute undue experimentation. In In re Wands, 8 USPQ2d 1400 (Fed. Cir. 1988), the court determined that there is no set level of occurrence or set number of screening assays that automatically qualifies as undue experimentation (the determination of undue experimentation "must be made in view of the circumstances of each case and cannot be made solely by reference to a particular numerical cutoff"). The court applied the factors enumerated in Ex parte Forman to the facts of the case and held that it would not require undue experimentation to obtain antibodies used in a claimed immunoassay taking into account the fact that "practitioners of [monoclonal antibody technology] are prepared to screen negative hybridomas in order to find one that makes the desired antibody." Id at 1406.

In this regard, Applicant submits that the generation and identification of artificial chromosomes does not require undue experimentation simply because a series of cell lines is analyzed in the process of obtaining a line that contains a desired artificial chromosome. Screening of transformant or fused cell lines, even in large numbers, using standard common methods such as Southern or *in situ* hybridization or LacZ staining is routine in a field such as molecular biology in which the level of skill is high. In addition, the instant application provides a great deal of guidance in terms of the detailed characteristics of the various artificial chromosomes and the screening techniques used to evaluate these characteristics that readily enables one of skill in the art to identify cell lines containing artificial chromosomes. Furthermore, the application describes (in Examples 2 through 7) numerous MAC-containing cell lines that were obtained by repeating the same artificial chromosome generation methods as taught in the specification, including, among others, cell lines EC3/7, EC3/7C5, EC3/7C6, KE1-2/4, TF1004G/19, TF1004G-19C5, TF1004G24, NHHL24, G3D5, G4D6, H1D3, 19C5xHa3, 19C5xHa4, 19C5xHa47, H1xHE41, 1B3 and 1B4.

Therefore, when the occurrence rates cited in Kereso et al. are interpreted in a reasonable manner, analysis considering the factors enumerated in Ex parte Forman leads to the conclusion that undue experimentation would not be required to obtain artificial chromosomes. Thus, contrary to the assertion set forth on page 8 of the Office Action, the MACs deposited by Applicant at the European Collection of Animal Cell Culture are not the only artificial chromosomes enabled by the application; rather the considerable direction and guidance provided in the application, the large number of exemplary artificial chromosome-containing cell lines and the standard screening procedures used in identifying artificial chromosomes enable a broad scope of artificial chromosome compositions.

3. MACs containing heterologous genes can be reliably produced and provide for high-level, copy number-dependent expression of the heterologous genes.

(a) Applicant's specification describes methods for incorporation of heterologous genes into MACs and contains numerous specific examples of such MACs

In the rejection of the claims 35 U.S.C. §112, first paragraph, it is alleged that there is no evidence that the artificial chromosomes disclosed in the above-captioned application can be used as a basis for introducing and expressing foreign genes in animals. In particular, it is stated that the specification teaches that one could introduce such sequences into MACs by *in vivo* homologous recombination into sequences present in the MACs such as phage λ sequences; however, the art is alleged to suggest that such homologous recombination would occur rarely, if at all, and the specification allegedly does not provide guidance as to how to accomplish such integration.

Applicant respectfully disagrees with the allegation that the specification does not provide guidance as to how to accomplish integration of heterologous DNA into artificial chromosomes. On the contrary, the specification teaches in detail how to generate artificial chromosomes containing foreign DNA and

provides multiple examples of such artificial chromosomes. For example, methods for generating artificial chromosomes as taught in the specification are based in the integration of heterologous DNA into a chromosome within the pericentric region. As described on page 5, lines 3-13, the heterologous DNA, which includes DNA encoding a selectable marker, is introduced into cells which are grown under selective conditions. Transformants that survive growth under selection have incorporated the foreign DNA. Thus, even if integration of the foreign DNA is not a high-frequency occurrence, and only a portion of the cells have incorporated the foreign DNA, those cells will be readily identified by growth on medium containing the selection agent. The surviving transformants are analyzed to identify those in which the heterologous DNA has integrated in the pericentric region of the chromosome thereby resulting in chromosomes with more than one centromere or that are fragments of chromosomes that had more than one centromere. Artificial chromosomes are then prepared from these chromosome fragments which contain the heterologous DNA.

Several specific examples of the **repeated** success of these methods are also provided in the specification. For example, as described in Example 2A beginning on page 45 of the specification, EC3/7 cells containing a neo-centromere and formerly dicentric chromosome were generated by contransfection of mouse LMTK⁻ fibroblast cells (i.e., a somatic cell line) with λ constructs λ CM8 and λ gtWESneo carrying human and bacterial DNA followed by growth of the transformants in the presence of neomycin. Integration of the heterologous DNA occurred in chromosome 7 and yielded a new centromere (neo-centromere) contained within a dicentric chromosome. Breakage of the dicentric chromosome yielded a fragment containing the neo-centromere and the formerly dicentric chromosome. *In situ* hybridization studies of these cells using a λ DNA probe **confirmed the presence of the integrated foreign DNA** at the neo-centromere and on the end of the formerly dicentric chromosome.

In another example (see Example 4 beginning on page 52 of the above-captioned application), a subclone of the EC3/7 cell line, i.e., cell line EC3/7C5, was transfected with λ phage DNA, pH132 (carrying the hygromycin B resistance gene and the anti-HIV-1 *gag* ribozyme gene) and pCH110 (carrying the β -galactosidase gene). Stable transformants were selected in the presence of high concentrations (400 μ g/ml) of hygromycin B. Analysis of one of the transformants, cell line TF1004G-19, by G-banding and *in situ* hybridization using λ CM8 (containing λ and human DNA) as a probe demonstrated that **integration had occurred** in the formerly dicentric chromosome 7 of the EC3/7C5 cell line which also contained a heterochromatic chromosome arm (i.e., a sausage chromosome). *In situ* hybridization analysis of a subclone (TF1004G-19C5) of this transformant using biotin-labeled λ DNA and subfragments of the hygromycin resistance and β -galactosidase genes as probes showed a hybridization signal only in the heterochromatic arm of the sausage chromosome, demonstrating that **these genes co-integrated** in the pericentric heterochromatin.

The specification, on pages 90-96, further describes the use of vectors for targeted introduction of heterologous DNA into artificial chromosomes. These examples teach site-specific integration of heterologous genes into artificial chromosomes through the use of homology targeting vectors. The heterologous gene of interest is subcloned into a targeting vector which contains nucleic acid sequences that are homologous to nucleotides present in the artificial chromosome. The vector is then introduced into cells containing the artificial chromosome for specific site-directed integration into the artificial chromosome through a recombination event at sites of homology between the vector and the chromosome. The homology targeting vectors may also contain selectable markers for ease of identifying cells that have incorporated the vector into the artificial chromosome as well as lethal selection genes that are expressed only upon extraneous integration of vector into the recipient cell

genome. Two exemplary homology targeting vectors, λ CF-7 and p λ CF-7-DTA, are described beginning on page 91 of the specification. Vector λ CF-7 contains the Charon 4A λ left arm, the CFTR encoding DNA linked to the CMV promoter and SV40 polyA signal, the ura3 gene, the puromycin resistance gene linked to the SV40 promoter and polyA signal and the Charon 4A λ right arm. The λ DNA fragments provide sequences homologous to nucleotides present in the exemplary artificial chromosomes. The vector is introduced into cells containing the artificial chromosomes exemplified herein. For example, introduction of a linearized λ CF-7 vector into megachromosome-carrying fusion cell lines, such as those described and specifically exemplified in detail in the specification, provides for specific integration into the megachromosome through recombination between the homologous bacteriophage λ sequences of the vector and the artificial chromosome.

An independent example of successful integration of heterologous DNA into a human minichromosome derived from human chromosome 9 is provided in Raimondi et al. [(1996) Human Gene Ther. 7:1103-1109.], a copy of which is attached hereto. Raimondi et al. report the introduction of a selectable marker gene, i.e., the neomycin resistance gene, into a human supernumerary minichromosome by co-transfection of a hybrid cell containing the minichromosome with the neomycin-resistance gene and chromosome 9-specific α satellite DNA. *In situ* hybridization using neomycin resistance gene and α satellite DNA probes demonstrated the foreign DNA integrated into the centromeric DNA of the minichromosome. Thus, just as taught in the above-captioned application, integration of foreign DNA into a chromosome was achieved using standard transfection and selection techniques. It is noted that this report published three months after the date of filing of U.S. patent application serial no. 08/629,822 to which the above-captioned application claims the benefit of priority; however, it is not provided to demonstrate the state of the art at the time of filing of the application. Rather, Applicant submits

this report simply as yet another example, in addition to those provided in the instant application, of the reliability of the exact methods described in the specification in the successful integration of heterologous DNA in extra-genomic chromosomes.

These examples demonstrate that, following the teaching of the specification, and using standard recombinant DNA procedures of transfection and selection, artificial chromosomes containing heterologous genes may be obtained through the integration of foreign DNA into chromosomal DNA. Irrespective of any suggestion in Brown *et al.* [(1996) *Curr. Opin. Genet. Devt.* 6:281-288] to the contrary (as mentioned on page 9 of the Office Action), the integration of foreign DNA in these examples occurred in somatic cell lines without the need for shuttling of chromosomes into an alternative cellular host. Although Brown *et al.* may suggest that the frequency of homologous recombination in mammalian somatic cells is too low for manipulation of mini-chromosomes, it provides no basis for this generalization. Brown *et al.* provides not one piece of data from which to draw this conclusion nor does it cite to the data or conclusions of others.

- (b) **The results of multiple different assays designed to detect and quantitate the functional heterologous gene product in cells containing MACs demonstrate stable, high-level expression of heterologous genes in artificial chromosomes.**

It is further alleged in the Office Action that it is unpredictable whether heterologous genes contained in MACs would be expressed, particularly in view of the fact that the inserted sequences would be imbedded in heterochromatic sequences and that the specification does not provide quantitative information regarding the level of foreign gene expression in an artificial chromosome carrying the β -galactosidase gene. It is also asserted in the Office Action that, of the disclosed MACs, only the sausage chromosome in TF1004G-19 was shown to express the foreign β -gal sequence.

In response, it is respectfully submitted that the application discloses numerous specific examples of expression of a heterologous gene within artificial chromosomes of all types, including expression from genes contained within the heterochromatic environment of a satellite artificial chromosome. First, each and every one of the artificial chromosome-containing cell lines specifically exemplified in the application (including cell lines deposited with the European Collection of Animal cell Culture) is direct evidence of significant levels of expression of foreign genes carried in the artificial chromosomes within the cells. The very existence of these artificial chromosome-containing cell lines is attributable to the expression of heterologous selectable marker genes contained in the artificial chromosomes which enabled the cells to survive growth on high levels of toxic antibiotic selective agents (e.g., neomycin, hygromycin, puromycin) by conferring a resistant phenotype to the cells.

Second, contrary to the Examiner's assertion that β -galactosidase expression in the TF1004G-19 sausage chromosome-containing cell line was identified by *in situ* hybridization but was not quantified, Example 4 (beginning on page 52) of the application describes the results of an extensive analysis of heterologous gene expression in this cell line. Structural analysis of the sausage chromosome in subclone TF1004G-19C5 through *in situ* hybridization with biotin-labeled fragments of the hygromycin resistance gene and the β -galactosidase gene revealed that it contains a ~100-150 Mb heterochromatic arm composed of four to five satellite segments rich in satellite DNA, and evenly spaced integrated heterologous DNA sequences. TF1004G-19C5 cells grew very well in the presence of 200 μ g/ml or even 400 μ g/ml hygromycin B, thereby providing evidence of substantial expression of the hygromycin resistance gene. The expression of the non-selected β -galactosidase gene in the TF1004G-19C5 transformant was determined by LacZ staining of the cells. By this method, one hundred percent of the cells stained dark blue, showing that

there is a high level of β -galactosidase expression from the sausage chromosome in all of the cells.

Satellite artificial chromosomes, by nature of the extensive, megabase-size array of repeated amplicons contained therein, each of which contains multiple copies of the heterologous gene, are capable of providing heterologous gene expression at unprecedented levels since traditional plasmid-based vectors are restricted in size and therefore copy number of heterologous genes. As discussed above, Example 8 (beginning on page 68) of the application describes this unique architecture of a satellite artificial chromosome, i.e., a megachromosome of H1D3 cells, as elucidated by BrdU pulse labelling and immunolabelling experiments. As depicted in Figure 3 of the instant application, the foreign DNA contained within the megachromosome was determined to be present in high copy numbers located at each end of each ~30 Mb amplicon. Thus, satellite artificial chromosomes are uniquely equipped to provide the capacity for multiple copies of heterologous genes and high-level expression thereof.

It is noted that on page 8 of the Office Action a question is raised as to the use of a transgenic animal whose cells express a β -galactosidase gene contained in a sausage chromosome such as that within TF1004G-19 cells. The relevance of this inquiry is unclear as none of the claims is directed to a transgenic animal expressing β -galactosidase. It is well understood in the art that the β -galactosidase gene is commonly used as a reporter gene in methods involving introduction of DNA into eukaryotic cells because the level of expression of the enzyme encoded by the gene is readily determined using simple assays. The sausage chromosome of the TF1004G-19 cells additionally contains the anti-HIV ribozyme and the hygromycin phosphotransferase gene, and the β -galactosidase gene is an indicator of expression of those genes as well.

The introduction of MACs into embryos or ES cells

It is noted on pages 10-11 of the Office Action that certain of the claims directed to methods for producing a transgenic animal comprise a step that specifies that an artificial chromosome is introduced into an embryonic stem cell. It is alleged that the state of the art supports that only mouse ES cells were available for production of transgenic animals because the prior and post-filing art are replete with references which indicate that ES cell technology is generally limited to the mouse system and that only "putative" ES cells exist for other species.

As stated above, in order to satisfy the enablement requirement of 35 U.S.C § 112, first paragraph, the specification must teach one of skill in the art to make and use the invention without undue experimentation. Atlas Powder Co. v. E.I. DuPont de Nemours, 750 F.2d 1569, 224 USPQ 409 (1984). This requirement can be satisfied by providing illustrative examples or terminology. This is not meant, however, to require "a specific example of everything *within the scope* of a broad claim." In re Anderson, 176 USPQ 331, at 333 (CCPA 1973), emphasis in original.

The specification describes numerous methods for the introduction of artificial chromosomes into cells, tissues and animals and cites extensive references that detail techniques used in such methods. Included in these descriptions are references to published protocols used in embryonic stem cell technology, for example: Hogan et al. (1994) *Manipulating the Mouse Embryo, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, see, especially, pages 255-264 and Appendix 3; U.S. Patent No. 5,453,357; Guide to Techniques in Mouse Development in Methods in Enzymology Vol. 25, Wassarman and De Pamphilis, eds. (1993), pages 803-932.

It is clear from the disclosure in the specification, including specific examples of the manipulation of exemplary artificial chromosomes in accordance

with standard recombinant DNA manipulation techniques, that the artificial chromosomes provided therein are contemplated for use in a wide variety of applications, including the production of transgenic animals, employing any known procedure used with any heterologous gene and vector system from any source. Based on the extensive teaching in the specification of methods of making, identifying, isolating and manipulating artificial chromosomes, it would not require undue experimentation for one skilled in the art to generate artificial chromosomes and utilize them in established procedures for developing transgenic animals. Methods of developing transgenic animals through initial introduction of transgenes into embryonic stem cells are known in the art regardless of the number of species of embryonic stem cells that may exist. Therefore, it is respectfully submitted that a claim to a method for producing a transgenic animal comprising a step of introducing an artificial chromosome (i.e., a satellite artificial chromosome or a minichromosome as disclosed in the subject application) into an embryonic stem cell is directed to subject matter supported in the specification in accordance with the requirements of 35 U.S.C. §112, first paragraph.

The Examiner suggests that 'ES cell technology is *generally* limited to the mouse system and that only "*putative*" ES cells exist for other species' (emphasis added). It is noted that the statement is equivocal and does not appear to be fairly supported by the excerpts of the cited documents selected by the Examiner and relied on in reaching the broad conclusion that "the state of the art supports that only mouse ES cells were available for use for the production of transgenics." Clearly, this could not have been the case since Mullins *et al.* (which the Examiner has provided) state that bovine ES cells could be generated (page S38, left column) and that chimeric animals have been developed from pigs using swine ES cells (page S38, left column), and Seamark (also provided by the Examiner) states that the demonstration of the feasibility

of the chimaeric route for reinstating an ES cell genome into the germ line of the pig is a major advance (see abstract).

In light of the extensive teaching in the specification of methods of making, identifying and isolating artificial chromosomes and of introducing artificial chromosomes into a vast array of host cells, it would be unduly limiting to require limitation of the claims based on species of ES cells, particularly in view of the equivocal nature of the grounds for the rejection.

As additional grounds for the rejection of the claims under 35 U.S.C. §112, first paragraph, it is alleged in the Office Action that the art suggests that it is unpredictable whether large MACs could be delivered into embryo or ES cells by any method, and the specification does not exemplify the successful delivery of such a DNA molecule or provide guidance how to do so. In support of this assertion, the Examiner relies on publications by Lamb *et al.* [(1995) *Curr. Opinion: Genetics & Dev.* 6:342-348] and Huxley [(1994) *Gene Therapy* 1:7-12] which indicate that the efficiency of introduction of large-sized DNA molecules into embryonic cells is generally lower than it is for introduction of smaller DNA molecules and that difficulties due to DNA shearing and fragmentation may be encountered. Applicant respectfully disagrees with an assessment of the art as suggestive of unpredictability of the claimed methods and also with an assessment of the specification as lacking guidance for the following reasons.

First, as discussed above in the section describing the state of the art at the time of filing of the above-referenced application, there was a considerable body of knowledge with respect to DNA manipulation techniques available at the time the application was filed. The specification teaches that the artificial chromosomes disclosed therein may be introduced into cells, e.g., ES cells, using methods such as cell fusion, microinjection, lipofection, microcell fusion, electroporation, microprojectile bombardment or direct DNA transfer. At the time the application was filed, the art provided detailed protocols for carrying

out these techniques of DNA transfer, many of which are referenced in the above-captioned application (see the section above regarding the state of the art). Accordingly, it is respectfully submitted that the specification provides ample guidance as to how to deliver artificial chromosomes into cells such as embryos and ES cells.

Furthermore, although the Examiner cites Lamb *et al.* and Huxley as suggestive of lower transfer efficiencies for larger DNA molecules as compared to smaller DNA molecules, there is no indication that large DNA molecules cannot be successfully introduced into cells such as ES cells. The rejected claims are directed to a method of producing a transgenic animal, not to a "high-efficiency" method for producing a transgenic animal. There is no requirement of high efficiency for patentability. It is required only that any amount of experimentation used by one of skill in the art in making and using the claimed invention not be undue.

The specification teaches that artificial chromosomes may be introduced into cells such as ES cells using a variety of DNA transfer methods. The specification further teaches how to determine if cells that have received the artificial chromosomes have incorporated them in functional form. For example, as described on page 105, lines 12-29, the recipient cells may be analyzed for expression of a selectable marker gene, such as the β -galactosidase gene. Additionally, the offspring of the surrogate mother female animal into which the artificial chromosome-containing embryonic cells are implanted can be analyzed in several ways for the presence and expression of the transgenes contained in the artificial chromosome (see, for example, pages 111-113 of the specification). Thus, although the transfer of artificial chromosomes into embryos or ES cells may not necessarily always be with high efficiency, it is possible to screen the recipient cells and determine which have incorporated the artificial chromosomes without undue experimentation.

Instances of the successful transfer of large, megabase-sized DNA molecules into embryonic cells have been reported. For example, human chromosome fragments containing more than 10 Mb of DNA have been microinjected into the pronuclei of fertilized mouse eggs, and the resulting embryos were analyzed by *in situ* hybridization to determine whether the human chromosomal DNA was maintained in the developing embryo [see Richia and Lo (1989) Science 245:175-177, a copy of which was provided to the USPTO on June 24, 1996 with an Information Disclosure Statement submitted in connection with parent application serial no. 08/629,822]. Hybridization signals were observed in half of the embryos analyzed. Thus, not only does the art suggest the transfer of megabase-sized DNA into embryonic cells, it conclusively *demonstrates* that such DNA transfer can be and has been successfully carried out.

In addition, microcell fusion techniques, such as described in the instant application (see e.g., page 33, lines 22-28, page 42, lines 7-21, page 81, lines 12-30 and page 110, lines 18-20), have been used to successfully transfer human chromosomes into mouse ES cells (see Tomizuka et al. (1997) Nature Genetics 16:133-141 a copy of which is attached hereto). The fused ES cells were then injected into embryos which were transferred into pseudopregnant mice. Transgenic mice resulting from the embryos expressed genes contained in the human chromosomes. Thus, just as taught in the above-captioned application, chromosome-size DNA may be transferred into ES cells using microcell fusion techniques. It is noted that this report published after the date of filing of the above-captioned application; however, it is not provided to demonstrate the state of the art at the time of filing of the application. Rather, Applicant submits this report simply as yet another example, in addition to those provided in the instant application, of the reliability of the exact methods described in the specification in the successful transfer of megabase-sized DNA into embryonic cells.

It is also alleged in the Office Action that the specification provides no guidance as to how much DNA would be required to generate a transgenic animal and whether the necessary amount could be purified and delivered. It is respectfully submitted that the specification provides ample guidance for the purification and delivery of artificial chromosomes as may be used in the generation of transgenic animals.

For example, on page 23, lines 8-12, the specification teaches that metaphase artificial chromosomes may be obtained, such as by addition of colchicine to cells containing the artificial chromosomes, and the chromosomes purified by addition of AT and GC specific dyes on a dual laser beam based cell sorter. Preparative amounts of chromosomes (2-3 mls of 10^6 chromosomes/ml) at a purity of 95% or higher can be thereby obtained and used for delivery to cells. On page 30, line 24, through page 31, line 8, the specification describes a specific method for isolation of artificial chromosomes that is provided in the application. This method is based on fluorescence activated cell sorting [FACS] and takes advantage of the nucleotide base content of the artificial chromosomes, and in particular satellite artificial chromosomes, which differs from any other chromosomes in a cell. In these methods, metaphase chromosomes are isolated and stained with base specific dyes, such as Hoechst 33258 and chromocycin A3. These procedures are described in detail in Example 10, beginning on page 78 of the specification.

Additionally, Example 14 of the instant application describes in detail the delivery, e.g., through microinjection or microcell fusion, of artificial chromosomes into embryos or ES cells. In particular, on page 110, lines 3-6, the specification provides an exemplary protocol in which fertilized mouse embryos are microinjected with megachromosomes (1-10 pL containing 0-1 chromosomes/pL) isolated, for example using the purification procedure as disclosed in the application, from a megachromosome-containing cell line. Based on these teachings, one of skill in the art may empirically determine

appropriate amounts of artificial chromosomes for use in a particular transfer procedure.

The generation of transgenic animals in which a heterologous gene is stably maintained and efficiently expressed.

It is alleged in the Office Action that it is not clear whether MACs would be maintained under *in vivo* conditions, or whether MACs bearing large DNA inserts would be maintained stably and in unarranged form. It is further alleged that it is not predictable whether MACs with centromeres of mammalian origin would function in other species or whether MACs could be maintained in the germline. In response to this allegation, it is respectfully submitted that Applicant has provided ample demonstration of the stable maintenance and broad-range applicability of artificial chromosomes in the generation of transgenic animals.

The specification provides data demonstrating stable *in vitro* maintenance of the artificial chromosomes in cells and the Office Action fails to provide an objective basis for doubting the stability of the artificial chromosomes *in vivo*

As described throughout the specification, numerous artificial chromosome-containing cell lines have been generated and maintained in culture for extended periods of time. A number of these cell lines have in fact been deposited at the European Collection of Animal cell Culture, as disclosed in the application. The artificial chromosomes within many of these cell lines have been extensively studied by multiple types of analyses for structural and functional stability.

For example, as described on page 47, lines 11-15, long-term analysis of EC3/7C5 and EC3/7C6 cell lines (140 and 128 metaphases, respectively) revealed that minichromosomes were present in 97.2% and 98.1% of the cells, respectively. The minichromosomes have been maintained for over 150 cell generations. On page 49, lines 19-24, the specification describes the results of further analysis of the EC3/7C5 cell line grown under both selective and non-

selective conditions. EC3/7C5 cells grown in non-selective medium for 284 days and then transferred to selective medium containing 400 μ g/ml G418 showed a 96% plating efficiency (colony formation) compared to control cells cultured permanently in the presence of G418. Cytogenetic analysis indicated that the minichromosome is stably maintained at one copy per cell both under selective and non-selective culture conditions.

The Examiner has provided no objective, independent, reasonable basis for doubting that the stability of MACs as demonstrated *in vitro* can be extrapolated to equivalent stability *in vivo*. Instead, it is asserted on page 12 of the Office Action that it is not predictable what modifications would occur *in vivo* "given the degree to which cultured EC3/7C5 cells give rise to modified forms of MACs." The basis for this assertion is unclear. As described above, extended analysis of the EC3/7C5 cell line revealed that the minichromosome contained therein is structurally and functionally stable. It is only with extrinsic intervention through the imposition of certain very specific conditions that it is possible to generate satellite artificial chromosomes from the formerly dicentric chromosome in EC3/7C5 cells. Modified artificial chromosomes do not arise spontaneously in the EC3/7C5 cell line in culture. Instead, satellite artificial chromosomes may be generated only by following the methods disclosed in the specification, such as by subsequent transfection with additional foreign DNA and imposition of certain selective conditions, which are not conditions that occur *in vivo*.

The specification provides evidence of the cross-species functioning of the artificial chromosomes and the Office Action fails to provide objective basis for asserting unpredictability of such functioning.

It is further alleged in the Office Action that it is unpredictable whether MACs bearing centromeres from mouse or human would function in organisms such as fish, insects, reptiles, amphibians, arachnids or unspecified mammals, as suggested in Claim 40. Additionally, it is alleged that there is no disclosure

in the art of transgenic reptiles or arachnids, as recited in claim 40, and the specification does not teach how to generate such animals.

Claim 40 has been amended to specify that the transgenic animal is a fish, insect, amphibian, bird or mammal. The amendment of Claim 40 herein should not be construed as acquiescence in the Examiner's allegation; rather, the amendment was included at this time to focus the issues in the interest of advancing prosecution with the intent of pursuing claims further claims in a continuing application.

It appears that the assertion in the Office Action of an alleged unpredictability of MACs bearing mouse or human centromeres functioning in other organisms is based on the Examiner's remark concerning how little is understood about the structure and function of centromeres from any species. No specific independent evidence in support of this remark is provided. It is respectfully submitted, however, that the level of understanding of centromere structure and function is not relevant to the the question of whether the claimed methods and animals are enabled for multiple species. First, irrespective of the level of understanding of centromeres, evidence exists for the cross-species functioning of centromeres. For example, each of the 23 human chromosomes has been successfully transferred into mouse or hamster cells in which they are maintained and functional. Additionally, as noted above, Tomizuka et al. [(1997) Nature Genetics 16:133-141] have generated transgenic mice by injecting embryos with ES cells into which human chromosomes had been transferred by microcell fusion. The transgenic mice resulting from the embryos expressed genes contained in the human chromosomes.

As discussed above, with respect to the artificial chromosomes disclosed in the subject application, there is ample evidence that MACs based in one mammalian species function in another mammalian species. Furthermore, the application describes methods for transfer and expression of mammalian artificial chromosomes in insect cells. For example, as described on page 84, insect cells

may be fused with mammalian cells containing minichromosomes and/or satellite artificial chromosomes using a calcium/PEG cell fusion protocol followed by selection of heterokaryons to obtain insect cells containing an artificial chromosome as reflected by expression of a phenotype conferred by a gene contained within the artificial chromosome.

In addition, the application teaches that the methods of generating MACs are broadly applicable to any mammalian, as well as other animal, species. Species-specific artificial chromosomes for use in the production of a transgenic animal of the same species certainly would be expected to function in the transgenic animal.

It is also alleged that, generally, transgene behavior cannot be extrapolated from one species of animal to another. In support of this assertion, reference is made to (1) Wall [(1996) *Theriogenology* 45:57-68] which allegedly discloses the unpredictability of transgene behavior due to factors such as position effect and unidentified control elements (2) Kappel et al. (JE) which allegedly discloses the existence of cellular mechanisms that may alter the pattern of gene expression resulting from differential CpG methylation and (3) Strojek and Wagner (NF) which allegedly points out the unpredictability of expression in different species due to differential interaction of cis acting elements with trans-acting factors between species. It is concluded that given such species differences in the expression of a transgene, it would require undue experimentation to extend the results achieved in transgenic mice to the levels of transgene product in any other transgenic animal.

In response, it is first noted that the publications on which this aspect of the rejection is based appear, based on the characterization of them in the Office Action, to relate to possible differences in the expression of **specific** transgenes of **specific sequence** in different species. While this generally in any type of transgenic application may or may not be the case, the issue at hand appears to be whether the artificial chromosomes disclosed in the subject

application are able to effect *any* expression in *any* transgenic animal and if so, whether the artificial chromosomes can be applied to the generation of multiple species of transgenic animals. As already discussed at length above, Applicant provides ample guidance in the specification for practicing the claimed methods and achieving the claimed animals such that one of skill in the art following the teachings of the application and combining them with standard DNA manipulation techniques could practice the methods without undue experimentation.

Furthermore, it is of interest to note that the passage in the Wall publication to which the Examiner refers states that aberrant expression patterns in some transgenic animals results from random integration of transgenes into the host genome (i.e., the position effect). Thus, for example, if a transgene "lands near highly active genes, the transgene's behavior may be influenced by endogenous genes." The position effect is one of the main barriers to successful heterologous gene expression that is overcome by the use of the artificial chromosomes in the claimed methods of transgenic animal production. Because the artificial chromosomes are maintained as independent, extra-genomic elements in host cells, they eliminate the unpredictability associated with standard methods of introducing foreign genes into host cells via uncontrolled, random integration into the host genome.

It is further questioned in the Office Action whether MACs could be maintained in the germline since Brown et al. point out that male meioses are particularly sensitive to the presence of unpaired chromosomes and that an unpaired marker chromosome will often block male meiosis during the first division. Because none of the rejected claims specifically cites germline transmission of a MAC, it is respectfully submitted that this inquiry is not relevant to the rejection of the claims under 35 U.S.C. §112, first paragraph. Nonetheless, it is clear that it is not valid to generalize the finding of sensitivity of male meioses as reported by Brown et al. in order to support any doubts

regarding the maintenance of MACs in the germline. As reported by Tomizuka et al. (see copy of the article attached hereto), transgenic mice developed from embryos injected with ES cells fused with microcells containing human chromosomes were able to transmit the human chromosomes through the germline.

The transgenic animals

With reference to the rejection of Claim 42 (directed to a transgenic animal produced by the method of claim 32) under 35 U.S.C. §112, first paragraph, it is asserted on page 14 of the Office Action that in order for an artisan to know how to use a transgenic animal, that animal would have to express a phenotype. It is alleged that it is unpredictable whether a transgenic animal generated with a MAC would express a transgene, or whether the degree of expression would be sufficient to generate a phenotype in the animal. It is concluded in the Office Action that because the specification allegedly does not disclose the generation of any transgenic animal by using a MAC, and in particular does not disclose the phenotype of such an animal, it is not enabling for how to make and use any transgenic animal by this method.

It appears that the entire basis for the rejection of claims directed to methods of producing transgenic animals using artificial chromosomes and the animals produced thereby is an asserted unpredictability of expression of transgenes from an artificial chromosome in a transgenic animal. It is noted in the Office Action that case law has established that in terms of predictability, additional factors, such as teachings in pertinent references, will be available to substantiate any doubts that the asserted scope of objective enablement is in fact commensurate with the scope of protection sought and to support any demands based thereon for proof [see page 15 of the Office Action citing In re Marzocchi, 439 F.2d 220, 223-223, 169 USPQ 367, 368-370 (CCPA 1971)].

As described above, however, in each case in which a lack of predictability is alleged in the Office Action, there is either (1) no objective, independent, reasonable basis provided in support of the allegation, or (2) where a reference has been cited by the Examiner in an effort to substantiate doubts concerning enablement of the methods and animals, Applicant has provided objective data as described in either the specification or in published reports to support arguments that it would not require undue experimentation to practice the claimed methods.

For example, the Office Action provides no independent basis for the Examiner's assertion of doubt that of the stability of artificial chromosomes *in vivo*. Applicant, however, provides data in the subject specification of extended stable maintenance of the artificial chromosomes *in vitro* under non-selective conditions. Such data could not be construed as a mere "broad statement made in support of enablement." In contrast, the unsubstantiated statement of doubt in the Office Action in effect appears to be a broad statement of doubt.

Although it is stated in the Office Action [with reference to Henikoff (1990)(IK)] that it is unpredictable whether heterologous DNA in the artificial chromosomes would be expressed, Applicant provides data in several examples in the subject application of stable, substantial expression of heterologous genes in the artificial chromosomes. As described above, first, the very existence of each and every one of the artificial chromosome-containing cell lines specifically exemplified in the application (including cell lines deposited with the European Collection of Animal cell Culture) confirms expression of selectable marker genes contained in the chromosomes.

Second, contrary to the Examiner's assertion that β -galactosidase expression in the TF1004G-19 sausage chromosome-containing cell line was identified by *in situ* hybridization but was not quantified, Example 4 (beginning on page 52) of the application describes the results of an extensive analysis of heterologous gene expression in this cell line. TF1004G-19C5 cells grew very

well in the presence of 200 $\mu\text{g/ml}$ or even 400 $\mu\text{g/ml}$ hygromycin B, thereby providing evidence of substantial expression of the hygromycin resistance gene. The expression of the non-selected β -galactosidase gene in the TF1004G-19C5 transformant was determined by **LacZ staining** of the cells. By this method, one hundred percent of the cells stained dark blue, showing that there is a high level of β -galactosidase expression from the sausage chromosome in all of the cells.

The demonstrated **fact** of heterologous gene expression in the artificial chromosomes combined with their unique architecture (e.g., the extensive, megabase-size array of repeated amplicons contained therein, each of which contains multiple copies of the heterologous gene) is convincing proof that the artificial chromosomes are capable of providing heterologous gene expression at unprecedented levels since traditional plasmid-based vectors are restricted in size and therefore copy number of heterologous genes.

It is also alleged in the Office Action that the specification fails to teach or provide guidance for the production of a transgenic animal, including a transgenic mouse, whose genome comprises a MAC which exhibits proper chromosome behavior such that the transgene would be expressed because, for example, Jiewen *et al.* [(1996) *Theriogenology* 45:336] disclose that proper MAC behavior in a host is measured by its proper decondensation, replication, and segregation into the daughter blastomeres along with embryo cleavage and proper transgene expression, yet the specification fails to teach any of these critical parameters. In addition, it is alleged that Jiewen *et al.* teaches that chromosome transgenics **must** involve microcell fusion which is not taught in the specification for the production of transgenic animals.

It is respectfully submitted that the publication relied on for this aspect of the rejection has been mischaracterized in the Office Action. First, of Jiewen *et al.*, do not state that proper chromosome behavior in a host is measured in the cited ways, but rather that they *can be* a measure of proper chromosome behavior. Thus, if the specification does not precisely address each of the

factors referred to in Jiewen *et al.*, it does not mean that the specification fails to teach transgenic animal production using artificial chromosomes. As described in detail above, the specification teaches each aspect of the processes involved in the generation of transgenic animals using the artificial chromosomes and provides data in support of the processes. Furthermore, as also described above, the application discloses the use of microcell fusion in transferring the artificial chromosomes and also provides numerous references to published procedures for the basic microcell fusion methods. It is noted, however, that there appears to be no such statement in Jiewen *et al.* that chromosome transgenics must involve microcell fusion. The study described in Jiewen *et al.* involved microinjection of CHO cell chromosomes into mouse zygote pronuclei, and is characterized as showing that the chromosomes were in fact capable of decondensation.

The Declaration of Perez demonstrates that using methods described in the application and standard methods of DNA manipulation, it was possible to generate transgenic mice employing artificial chromosomes as disclosed in the application.

The DECLARATION of Perez provided herewith describes the generation of transgenic mice using methods and materials disclosed in the above-referenced application and standard methods as described in the DECLARATION. Transgenic mice were produced by microinjection of 60 Mb murine satellite DNA-based artificial chromosomes containing multiple copies of the *lacZ* (β -galactosidase) and *hph* (hygromycin phosphotransferase) genes into the pronucleus of mouse zygotes.

Successive steps in the process of transgenic mice production were analyzed, including the presence and expression of artificial chromosomes in preimplantation embryos and the presence and integrity of the artificial chromosomes in founder and progeny mice, and the results are presented in the DECLARATION. As described in the DECLARATION, fluorescence *in situ* hybridization (FISH) analysis of preimplantation embryos injected with satellite

DNA-based artificial chromosomes demonstrated that 44% of the analyzed embryos contained intact satellite DNA-based artificial chromosomes in 8-67% of the total cells analyzed for each positive embryo. In β -galactosidase staining assays of injected preimplantation embryos at various developmental stages, 31% of the analyzed embryos showed X-gal staining indicating the presence of a functional marker gene in the artificial chromosomes that was expressed in a mosaic pattern.

Seven percent of the mice born after implantation of injected embryos into pseudopregnant female mice were positive for the *hph* gene in nucleic acid amplification analyses of tail DNA. The presence of intact satellite DNA-based artificial chromosomes in mitogen-activated peripheral blood lymphocytes from a female transgenic founder was confirmed by FISH analysis. This analysis also revealed that the artificial chromosomes were maintained as discrete chromosomes in approximately 60% of the cells analyzed and that they had not integrated into the endogenous chromosomes.

Mating of the transgenic female founder with wild-type F1 males yielded progeny, 46% of which were positive for the presence of the *hph* gene in nucleic acid amplification assays of tail DNA. FISH analysis of peripheral blood lymphocytes from progeny carrying the satellite DNA-based artificial chromosomes revealed that intact artificial chromosomes were present in approximately 60% of the analyzed cells with no apparent translocation of the artificial chromosome DNA onto the host chromosomes.

The results of these analyses demonstrate that satellite DNA-based artificial chromosomes as described in the above-referenced application can be used in standard methods of transgenic animal generation to yield viable transgenic animals containing within their cells intact, heterologous gene-containing artificial chromosomes as autonomous, stably replicating, extra-genomic elements. Furthermore, the results of these analyses demonstrate that

the satellite DNA-based artificial chromosomes are transmitted through the germline.

THE REJECTION OF CLAIMS 32-44, 59 and 60 UNDER 35 U.S.C. §112, SECOND PARAGRAPH

Claims 32-44, 59 and 60 are rejected under 35 U.S.C. 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention. Applicant respectfully traverses the rejection of these based on the following remarks and the amendment of the claims herein.

Claims 32 and 43 are allegedly incomplete since the preamble recites "for producing a transgenic animal" and the only step of the method is the introduction of a satellite artificial chromosome into an embryonic cell or of a minichromosome into an animal cell. Claims 32 and 43 have been amended to include a step of exposing the cell containing the artificial chromosome to conditions whereby a transgenic animal is developed therefrom and are believed to be complete.

As the Examiner correctly noted, in claims 36 and 39, which were written as dependent on claim 32, there was no antecedent basis for "the product." Claims 36 and 39 has been amended to depend from claim 35 which provides antecedent basis for "the product."

As also correctly noted by the Examiner, in claim 37, which was written as dependent on claim 32, there was no antecedent basis for "the anti-HIV ribozyme." Claim 37 has been amended to depend from claim 36 which provides antecedent basis for "anti-HIV ribozyme."

Claim 39 is further rejected as allegedly being vague and indefinite in the recitation of "a plurality of antigens" and "a plurality of pathogens" because it is not clear how many antigens or pathogens are encompassed by the claim. It is respectfully submitted that the claim is not rendered vague merely by the use of the word "plurality" which is standard, accepted claim language known to mean

the presence of more than one of the things that it modifies. Claims are not read in a vacuum but instead are considered in light of the specification and the general understanding of the skilled artisan. Clearly, the method of claim 39 is aimed at the production of a transgenic animal that is less susceptible or resistant to pathogen-induced diseases which adversely affect the health of the animal. Accordingly, the metes and bounds of the claim are defined by the number and types of pathogens against which an immunoprotective response is desired for the generation of a less-susceptible condition of the animal.

Claim 40 is rejected as allegedly being indefinite in the recitation of "the transgenic animal is a ... amphibians." It is respectfully submitted that the basis for this rejection is rendered moot by the amendment of claim 40 herein which deletes the word "amphibians."

THE REJECTION OF CLAIMS 42 AND 64-68 UNDER 35 U.S.C. §102/103

Claims 42 is rejected under 35 U.S.C. §102(b) as allegedly being anticipated by Larsson *et al.* (1994) because Larsson *et al.* teach a transgenic mouse harboring a ribozyme transgene directed against mouse β 2M mRNA. It is asserted that the claim does not require the expression of a transgene and does not recite a phenotype that would distinguish the claimed animal from any other transgenic animal and therefore the claimed animal is anticipated by Larsson *et al.*

Reconsideration and withdrawal of this rejection is respectfully requested in view of the amendment of claim 42 herein. As amended, claim 42 recites that the transgenic animal comprises satellite artificial chromosomes. Larsson *et al.* do not teach or suggest such animals and thus the claimed animals cannot be anticipated by the cited reference.

CLAIM REJECTIONS - 35 U.S.C. §103

As pointed out by the Examiner, the application names joint inventors and is jointly owned. Applicant is aware of the obligation imposed by 37 C.F.R. §1.56 and is currently reviewing the pending claims in connection therewith and

to ascertain whether a petition pursuant to 37 C.F.R. §1.48(b) is appropriate. Any submissions required in this regard will be provided under separate cover.

THE REJECTION OF CLAIM 43 UNDER 35 U.S.C. §103(a)

Claim 43 is rejected under 35 U.S.C. §103(a) as being unpatentable over either WO95/32297 (Brown et al., 1995) or Farr et al. (1995), either of the preceding in view of Brown (1992). Specifically, it is alleged that it would have been obvious for a person of ordinary skill in the art at the time the invention was made to use the minichromosome taught by Brown et al. (1995) or Farr et al. as the basis for generating cells comprising MACs, with a reasonable expectation of success that the minichromosomes would enter the cells and replicate. Applicant respectfully traverses this rejection for the following reasons.

Claim 43

Claim 43, as amended, is directed to a method of producing a transgenic animal comprising steps that include introducing exogenous DNA into a cell, wherein included in the DNA is DNA encoding a selectable marker and DNA encoding a gene product or products, selecting a cell that comprises a minichromosome that contains a neo-centromere, the added DNA and euchromatin, introducing the minichromosome into an animal cell and exposing the animal cell to conditions whereby a transgenic animal develops therefrom. As is clear from the claim, the method of claim 43 involves *de novo* formation of a minichromosome; the minichromosome generated in the method of claim 43 is **not** produced by fragmentation of an existing chromosome to simply produce a smaller version thereof. In addition, the method of claim 43 includes a step in which DNA encoding a gene product(s) of interest is introduced into the cell for incorporation into the minichromosome thereby providing for the expression of the DNA in cells of the transgenic animal which contain the minichromosome.

The prior art

Brown et al. and Farr et al. disclose methods of breaking off portions of existing chromosomes within a cell which serve to decrease the size of the existing chromosome. Such methods are described as telomere-associated chromosome fragmentation. Thus, in this method of generating a "minichromosome," a new chromosome with a new centromere is not created; instead, an existing chromosome is made smaller but still retains a significant portion of its original DNA of heterogeneous composition as well as the original centromere.

Relevant law

In order to establish prima facie obviousness, the prior art must provide a motivation whereby one of ordinary skill in the art would have been led to do that which the applicant has done Stratoflex Inc. v Aeroquip Corp., 713 F.2d 1530, 1535, 218 USPQ 871, 876 (Fed. Cir. 1983). The mere fact that prior art may be modified as suggested by the Examiner does not make the modification obvious unless the prior art suggested the desirability of the modification (In re Gordon, 733 F.2d at 902, 221 USPQ at 1127). In addition, the combination of references or the cited reference with the knowledge of those of ordinary skill in the art must result in the claimed subject matter. Obviousness is tested by "what the combined teachings of the references would have suggested to those of ordinary skill in the art" In re Keller, 642 F.2d 413, 425, 208 USPQ 871, 881 (CCPA 1981), but it cannot be established by combining the teachings of the prior art to produce the claimed invention, absent some teaching or suggestion supporting the combination (ACS Hosp. Systems, Inc. v Montefiore Hosp. 732 F.2d 1572, 1577. 221 USPQ 329, 933 (Fed. Cir. 1984)). "To imbue one of ordinary skill in the art with knowledge of the invention in suit, when no prior art reference or references of record convey or suggest that knowledge, is to fall victim to the insidious effect of a hindsight syndrome wherein that which only the inventor taught is used against its

teacher" W.L. Gore & Associates, Inc. v. Garlock Inc., 721 F.2d 1540, 1553, 220 USPQ 303, 312-13 (Fed. Cir. 1983).

The differences between the method of claim 43 and the methods disclosed in Brown et al. and Farr et al. are not taught or suggested by the combination of the cited references.

The method of claim 43 for the production of transgenic animals is based on the generation of a minichromosome that is formed *de novo*. As described in the specification (see, for example, page 21, line 5, through page 22, line 3), the integration of exogenous DNA into the pericentric region of a chromosome results in the amplification of chromosomal and heterologous DNA to yield a dicentric chromosome containing a new additional centromere (i.e., the neo-centromere). Separation of the dicentric chromosome between the two centromeres yields a neo-minichromosome containing the neo-centromere and the multiple copies of the heterologous DNA. As described on pages 47-49, of the specification, the neo-minichromosome possesses a unique architecture wherein the arm of the minichromosome is made up of repeated units that contain multiple copies the heterologous DNA together with some of the chromosomal DNA. The unique structure of the *de novo*-formed neo-minichromosome is of a defined composition, i.e., repeat-after-repeat of the heterologous DNA-containing unit, which provides for enhanced levels of expression of any heterologous gene contained therein.

In contrast, the method of "minichromosome" formation disclosed in Brown et al. and Farr et al. absolutely requires the introduction of a very specific exogenous DNA sequence, i.e., telomeric sequence, into an existing chromosome. In this method of telomere-associated chromosome fragmentation, a terminal portion of the existing chromosome is simply broken off from the chromosome and replaced with the added telomeric sequence, thereby reducing the size of the existing chromosome. The smaller-size chromosome still contains the original centromere and a large portion of the

heterogenous chromosomal DNA of unknown composition. In contrast to the neo-chromosome described in the instant application, which through an amplification process that accompanies the generation of the neo-chromosome contains multiple repeats of heterologous DNA, any foreign DNA that may be contained in the reduced-size chromosome of Brown et al. or Farr et al. is not present in high copy number.

There is no suggestion in Brown et al. or Farr et al. of a method for generating a new centromere or a chromosome containing a new centromere and a heterologous DNA repeat unit-based structure. There is no suggestion in either of these prior art references of selecting minichromosomes containing a neo-centromere or multiple repeats of heterologous DNA-containing units because the telomere-associated chromosome fragmentation method disclosed in these references does not produce a neo-minichromosome. In fact, both Brown and Farr et al. teach away from the possibility of any method of generating a minichromosome other than telomere-associated chromosome fragmentation. For instance, Farr et al. states on page 5444 (left column) that there are two basic approaches to building a MAC: (1) cutting and pasting cloned functional elements and (2) the fragmentation of an existing chromosome. Farr et al. teaches that a major limitation of the former method is defining a piece of DNA which will be capable of acting as a mitotically functional centromere (page 5444, right column). Likewise, Brown et al. discloses only one method for obtaining a chromosome fragment that is based on dissecting a chromosome (see page 3, lines 10-14). Brown adds nothing more to the teachings of these two references but merely confirms the approach provided therein. For example, Brown states on page 480 (left column) that the observation that cloned telomeric DNA can be used systematically to fragment chromosomes in yeast suggests how a systematic approach to the functional dissection of mammalian chromosomes may be pursued. Therefore, not only do these references fail to even contemplate *de novo* formation of a neo-minichromosome

as taught in the instant application, they urge the use of telomere-directed dissection of existing chromosomes as the only feasible method of generating a minichromosome. As such, the instantly claimed methods are not taught or suggested by Farr et al., Brown et al., Brown or the combination thereof.

THE REJECTION OF CLAIMS 43, 59 and 60 UNDER 35 U.S.C. §103(a)

Claims 43, 59 and 60 are rejected under 35 U.S.C. §103(a) as being unpatentable over U.S. Patent No. 5,288,625 (Hadlaczky) in view of Brown (1992). Specifically, it is alleged that Hadlaczky differs from the claimed invention in that it does not explicitly teach isolating the minichromosomes and introducing them into animal cells. However, it is further alleged that at the time the invention was made, Brown had taught that MACs could allow novel developmental or metabolic pathways to be introduced genetically into cells. It is concluded that it would have been obvious for a person of ordinary skill in the art at the time the invention was made to use the minichromosome taught by Hadlaczky as the basis for generating cells comprising MACs with a reasonable expectation of success that the minichromosomes would enter the cells and replicate.

Applicant respectfully traverses this rejection based on the following reasons.

Claim 43, as amended, is directed to a method of producing a transgenic animal comprising steps that include obtaining a cell that comprises a minichromosome that contains a neo-centromere, the added DNA and euchromatin, introducing the minichromosome into an animal cell and exposing the animal cell to conditions whereby a transgenic animal develops therefrom. As is clear from the claim, the method of claim 43 specifically involves steps in which a minichromosome containing DNA encoding a gene product(s) of interest is introduced into a cell which is specifically treated in such a way that the a transgenic animal develops from the cell.

The prior art

Hadlaczky discloses minichromosome-containing cell lines and methods of generating the cell lines. There is, however, no teaching in Hadlaczky of using the cell lines or methods of making the minichromosomes in the generation of a transgenic animal for the expression of gene products therein.

Brown, as described above, provides a general review of mammalian artificial chromosome (MAC) technology with the primary focus being the use of telomere-directed chromosome fragmentation as a possible means of generating MACs. Brown indicates that this method of dissecting existing chromosomes is preferred for the production of MACs.

The method of claim 43 is not taught or suggested by the combination of Hadlaczky and Brown.

The instant application discloses in detail and demonstrates the broad applicability of *de novo*-formed MACs and the methods of making the MACs in the production of transgenic systems. The specification teaches the many methods and manipulations involved in the functional transfer of heterologous DNA contained in these unique MACs for high-level expression in transgenic animals. Claim 43 is directed to a method as disclosed in the application for producing a transgenic animal comprising a minichromosome.

Hadlaczky teaches a method of generating a minichromosome and provides specific cell lines containing a minichromosomes. Methods of producing transgenic animals utilizing the minichromosomes are not taught or described in Hadlaczky.

Brown cannot be relied on as a supplement to Hadlaczky to arrive at a method of producing transgenic animals as instantly claimed. As described above, Brown urges the feasibility of using telomere sequences in the construction of a mammalian minichromosome (see page 480, right column). Brown does not contemplate *de novo* generation of a neo-minichromosome as taught in the instant specification. Furthermore, Brown does not teach methods

for the functional transfer of any MAC to any transgenic animal system to achieve high-level expression of heterologous gene contained therein.

It is respectfully submitted that there is no motivation or suggestion to combine the disclosure of Brown with that of Hadlaczký in order to arrive at the instantly claimed methods for producing transgenic animals. In fact, Brown would seem to strongly suggest the contrary in its characterization of the minichromosomes as disclosed in Hadlaczký. For instance, on page 483, bottom right column, to page 484, top left column, Brown refers to two publications of Hadlaczký describing the minichromosome as disclosed in U.S. Patent NO. 5,288,625 and states, with reference to the composition of sequence contained within the minichromosome, that it is "difficult to define the relationship of this DNA sequence to the functionally significant DNA at the mammalian centromere because the structure and sequence organization of this amplified array has not yet been determined." Therefore, Applicant respectfully submits that the inconsistent combination of Brown with Hadlaczký represents improper use of hindsight and does not support a rejection of claim 43 as obvious in view of the combined references.

Comments Regarding Information Disclosure Statements

It is noted that Applicant has been invited to particularly point out any references, other than those submitted with an Information Disclosure Statement on 6/9/99 which have been deemed non-analogous art, that may be either specifically analogous or definitely pertinent to the claimed invention. The Examiner refers to Golden Valley Microwave Foods Inc. v. Weaver Popcorn Co. Inc. for the proposition that a patent applicant's duty of disclosure includes a requirement of making such disclosure in a way as not to bury prior art references with other disclosures of less relevant prior art.

Applicant takes exception with any suggestion of noncompliance with the duty of disclosure. Applicant has diligently endeavored to comply with the duty to disclose information material to patentability as set forth under 37 C.F.R.

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AMENDMENT

§1.56 and interpreted by the U.S. Patent and Trademark Office in Section 2001.04 of the Manual of Patent Examining Procedures.

It is Applicant's understanding that, in accordance with Section 2001.04, patent applicants are advised to "submit information for consideration by the Office in applications rather than making and relying on their own determinations of materiality." This is precisely what Applicant has done.

As dictated by the Patent Rules in 37 CFR §1.97(h), the filing of an information disclosure statement shall not be considered to be an admission that the information cited in the statement is, or is considered to be, material to patentability as defined in 37 CFR 1.56. Furthermore, Applicant is unaware of any requirement in complying with the duty of disclosure to "particularly point out references that are definitely pertinent to the claimed invention" especially in view of the revision of the former rule that required applicants to provide a statement of the relevance of information listed in an information disclosure statement.

It is further respectfully submitted that the opinion of the District Court, N.D. Indiana, in its ruling in Golden Valley Microwave Foods Inc. v. Weaver Popcorn Co. Inc., 24 USPQ2d 1801, 1827 (1992), is misquoted on page 21 of the Office Action where it is stated that the patent applicant has a duty to make disclosure in such a way as not to "bury" prior art references within other disclosures of less relevant prior art. The court was not setting forth a requirement of any particular manner of disclosure in the duty to disclose, such as a requirement to "particularly point out references that are definitely pertinent to the claimed invention." Instead, the court simply held that it would be a violation of the duty of candor and fair dealing for an applicant or its attorney to bury a pertinent prior art patent reference in a series of disclosures of less relevant prior art references.

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Applicant and Applicant's representative strongly object to any suggestion that either party has buried pertinent prior art or is in any way in violation of the duty of candor. In fully disclosing references as provided with the Information Disclosure Statement submitted on 6/9/99, it was not, and is not, the intention of either party to bury prior art references. On the contrary, both Applicant and Applicant's representative have endeavored to be in complete compliance with the duty of disclosure as set forth in the Patent Rules, and in accordance with the guidelines provided in the Manual of Patent Examining Procedure, by providing the Office with all references for consideration rather than making and relying on their own determinations of materiality.

* * *

In view of the above amendments and remarks, reconsideration and allowance of the application are respectfully requested.

Respectfully submitted,
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

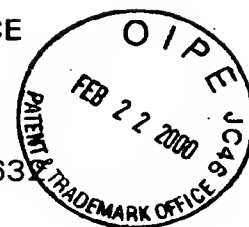
Applicant: HADLACZKY et al.

Serial No.: 09/096,648

Group Art Unit: 163

Filed: June 12, 1998

Examiner: Martin, J.



For: ARTIFICIAL CHROMOSOMES, USES THEREOF AND METHODS
FOR PREPARING ARTIFICIAL CHROMOSOMES

ATTACHMENTS

The following attachments are provided in connection with the
Amendment filed herewith.

1. A DECLARATION of PEREZ pursuant to 37 C.F.R. §1.132.

2. Copies of the following publications:

Klinger and Shin (1974). *Proc. Nat. Acad. Sci. U.S.A.* 71:1398-1402.

Rasko *et al.* (1979). *Cytogenet. Cell Genet.* 24:129-137.

Raimondi *et al.* (1996). *Human Gene Ther.* 7:1103-1109.

Tomizuka *et al.* (1997). *Nature Genetics* 16:133-143.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: HADLACZKY et al.

Serial No.: 09/096,648

Group Art Unit: 1632

Filed: June 12, 1998

Examiner: Martin, J.

For: ARTIFICIAL CHROMOSOMES, USES THEREOF AND METHODS
FOR PREPARING ARTIFICIAL CHROMOSOMES

ATTACHMENTS

The following attachments are provided in connection with the
Amendment filed herewith.

1. A DECLARATION of PEREZ pursuant to 37 C.F.R. §1.132.
2. Copies of the following publications:
 - Houdebine (1994) *J. Biotechnol.* 34:269-287
 - Drohan (1992) *J. Cell. Biochem.* 49:111-112
 - Wall *et al.* (1992) *J. Cell. Biochem.* 49:113-120
 - PCT Application Publication No. WO93/25567